

08-01-CO

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UTILITY PATENT APPLICATION TRANSMITTAL

*Submit an original and a duplicate for fee processing
(Only for new nonprovisional applications under 37 CFR §1.53(b))*

ADDRESS TO:

**Assistant Commissioner for Patents
Box Patent Application
Washington, D.C. 20231**

Attorney Docket No. 205965

First Named Inventor Crystal, Ronald G

Express Mail No. EG267442694US

G 09/07/00
U.S. PTO
629074
07/31/00

APPLICATION ELEMENTS

1. Utility Transmittal Form
2. Specification (including claims and abstract) [Total Pages 15]
3. Drawings [Total Sheets]
4. Combined Declaration and Power of Attorney [Total Pages]
 a. Newly executed
 b. Copy from prior application
[Note Box 5 below]
 i. Deletion of Inventor(s) Signed statement attached deleting inventor(s) named in the prior application
5. Incorporation by Reference: The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under Box 4b is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.
6. Microfiche Computer Program
7. Nucleotide and/or Amino Acid Sequence Submission
 - a. Computer Readable Copy
 - b. Paper Copy
 - c. Statement verifying above copies

ACCOMPANYING APPLICATION PARTS

8. Assignment Papers (cover sheet and document(s))
9. Power of Attorney
10. English Translation Document (if applicable)
11. Information Disclosure Statement (IDS)
 - Form PTO/SB/08A/B
 - Copies of References
12. Preliminary Amendment
13. Return Receipt Postcard (Should be specifically itemized)
14. Small Entity Statement(s)
 - Enclosed
 - Statement filed in prior application; status still proper and desired
15. Certified Copy of Priority Document(s)
16. Other:
Print EFS Bibliographic Data Output

17. If a CONTINUING APPLICATION, check appropriate box and supply the requisite information in (a) and (b) below:

(a) Continuation Divisional Continuation-in-part of prior application No. , filed . Prior application information: Examiner: ; Group Art Unit:

(b) Preliminary Amendment: Relate Back - 35 USC §120. The Commissioner is requested to amend the specification by inserting the following sentence before the first line:
 "This is a continuation divisional of copending application(s)

Serial No. , filed on

International Application, filed on , and which designates the U.S."

APPLICATION FEES

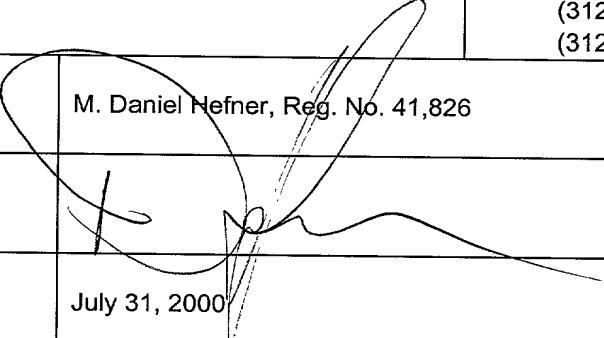
BASIC FEE					\$ 690.00
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total Claims	25 - 20 =	5	x \$18.00	\$ 90.00	
Independent Claims	3 - 3 =	0	x \$78.00	\$ 0.00	
<input type="checkbox"/> Multiple Dependent Claims(s) if applicable			+ \$260.00	\$ 0.00	
Total of above calculations =				\$ 780.00	
Reduction by 50% for filing by small entity =				\$ 390.00	
<input type="checkbox"/> Assignment fee if applicable			+ \$40.00	\$ 0.00	
TOTAL =				\$ 390.00	

UTILITY PATENT APPLICATION TRANSMITTAL

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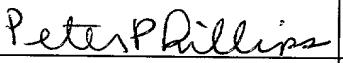
19. Please charge my Deposit Account No. 12-1216 in the amount of \$ 390.00.
20. A check in the amount of \$ _____ is enclosed.
21. The Commissioner is hereby authorized to credit overpayments or charge any additional fees of the following types to Deposit Account No. 12-1216:
- a. Fees required under 37 CFR §1.16.
 - b. Fees required under 37 CFR §1.17.
22. The Commissioner is hereby generally authorized under 37 CFR §1.136(a)(3) to treat any future reply in this or any related application filed pursuant to 37 CFR §1.53 requiring an extension of time as incorporating a request therefor, and the Commissioner is hereby specifically authorized to charge Deposit Account No. 12-1216 for any fee that may be due in connection with such a request for an extension of time.

23. CORRESPONDENCE ADDRESS

<input type="checkbox"/> Customer Number:	<input checked="" type="checkbox"/> M. Daniel Hefner, Reg. No. 41,826 Leydig, Voit & Mayer, Ltd. Two Prudential Plaza, Suite 4900 180 North Stetson Chicago, Illinois 60601-6780 (312) 616-5600 (telephone) (312) 616-5700 (facsimile)
Name	M. Daniel Hefner, Reg. No. 41,826
Signature	
Date	July 31, 2000

Certificate of Mailing Under 37 CFR §1.10

I hereby certify that this Utility Patent Application Transmittal and all accompanying documents are being deposited with the United States Postal Service "Express Mail Post Office To Addressee" Service under 37 CFR §1.10 on the date indicated below and is addressed to: Assistant Commissioner for Patents, Box Patent Application, Washington, D.C. 20231.

Peter Phillips		July 31, 2000
Name of Person Signing	Signature	Date

PATENT
Attorney Docket No. 205965

Applicant or Patentee: Crystal et al.
Application or Patent No.
Filed or Issued: July 31, 2000
For: METHOD OF ENHANCING BONE DENSITY

VERIFIED STATEMENT (DECLARATION)
CLAIMING SMALL ENTITY STATUS
37 C.F.R. §§ 1.9(f) & 1.27(d) - NONPROFIT ORGANIZATION

I hereby declare that I am an official empowered to act on behalf of the nonprofit organization identified below:

Name of Organization: Cornell Research Foundation, Inc.*
Address of Organization: 20 Thornwood Drive, Suite 105
Ithaca, New York 14850

Type of Nonprofit Organization:

- University or other institution of higher education.
 Tax exempt under Internal Revenue Service Code (26 U.S.C. §§ 501(a) and 501(c)(3)).
 Nonprofit scientific or educational organization under statute of state of the United States of America:
 Name of State: _____
 Citation of Statute: _____
 Would qualify as tax exempt under Internal Revenue Service Code (26 U.S.C. §§ 501(a) and 501(c)(3)) if located in the United States of America.
 Would qualify as nonprofit scientific or educational organization under statute of state of the United States of America if located in the United States of America.
 Name of State: _____
 Citation of Statute: _____

I hereby declare that the nonprofit organization identified above qualifies as a nonprofit organization as defined in 37 C.F.R. § 1.9(e) for purposes of paying reduced fees under Sections 41(a) and (b) of Title 35, United States Code, with regard to the invention entitled METHOD OF ENHANCING BONE DENSITY, by the inventors Ronald G. Crystal, Chisa Hidaka, Oheneba Boachie-Adjei, Bernard A. Rawlins, and Imre Kovacs, as described in:

- The specification filed herewith.
 Application No. , filed .
 Patent No. , issued .

I hereby declare that rights under contract or law have been conveyed to and remain with the nonprofit organization with regard to the above-identified invention.

*Cornell Research Foundation, Inc., is a Corporation which is wholly owned by Cornell University handling Patents and Licensing.

In re Application of Crystal et al.
Attorney Docket No. 205965

Others Having Rights In The Invention

If the rights held by the nonprofit organization are not exclusive, each individual, concern or organization having rights to the invention is listed below, and no rights to the invention are held by any person, other than the inventor, who would not qualify as an independent inventor under 37 C.F.R. § 1.9(e) if that person made the invention, or by any concern which would not qualify as a small business concern under 37 C.F.R. § 1.9(d), or a nonprofit organization under 37 C.F.R. § 1.9(e). (NOTE: Separate verified statements are required from each named person, concern, or organization having rights to the invention averring to his/her/its status as a small entity.)

Name: The Hospital for Special Surgery
Address: 535 East 70th Street
New York, New York 10021

Individual Small Business Concern Nonprofit Organization

Name: GenVec, Inc.
Address: 65 West Watkins Mill Road
Gaithersburg, Maryland 20878

Individual Small Business Concern Nonprofit Organization

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 C.F.R. § 1.28(b)).

I hereby declare that all statements made herein of my own knowledge are true, that all statements made on information and belief are believed to be true, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Name of Person Signing: James A. Severson
Title in Organization: President
Address of Person Signing: 20 Thornwood Drive, Suite 105
Ithaca, New York 14850


Signature

July 31, 2000

Date

PATENT
Attorney Docket No. 205965

Applicant or Patentee: Crystal et al.
Application or Patent No.
Filed or Issued: July 31, 2000
For: METHOD OF ENHANCING BONE DENSITY

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 Nonprofit scientific or educational organization under statute of state of the United States of America:
Name of State: _____
Citation of Statute: _____
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 Would qualify as nonprofit scientific or educational organization under statute of state of the United States of America if located in the United States of America.

Name of State: _____
Citation of Statute: _____

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- The specification filed herewith.
 Application No. , filed
 Patent No. , issued

I hereby declare that rights under contract or law have been conveyed to and remain with the nonprofit organization with regard to the above-identified invention.

In re Application of Crystal et al.
Attorney Docket No. 205963

Others Having Rights In The Invention

If the rights held by the nonprofit organization are not exclusive, each individual, concern or organization having rights to the invention is listed below, and no rights to the invention are held by any person, other than the inventor, who would not qualify as an independent inventor under 37 C.F.R. § 1.9(c) if that person made the invention, or by any concern which would not qualify as a small business concern under 37 C.F.R. § 1.9(d), or a nonprofit organization under 37 C.F.R. § 1.9(e). (NOTE: Separate verified statements are required from each named person, concern, or organization having rights to the invention averring to his/her/its status as a small entity.)

Name: Cornell Research Foundation, Inc.
Address: 20 Thornwood Drive, Suite 105
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Individual Small Business Concern Nonprofit Organization

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I hereby declare that all statements made herein of my own knowledge are true, that all statements made on information and belief are believed to be true, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Name of Person Signing:

Title in Organization:

Address of Person Signing: 535 East 70th Street
New York, New York 10021

Paddy Miller PADDY MILLER
EXECUTIVE VICE PRESIDENT

Paddy Miller
Signature

July 31, 2000

Date

PATENT
Attorney Docket No. 205965

Applicant or Patentee: Crystal et al.

Application or Patent No.

Filed or Issued: July 31, 2000

For: METHOD OF ENHANCING BONE DENSITY

VERIFIED STATEMENT (DECLARATION)
CLAIMING SMALL ENTITY STATUS
37 C.F.R. §§ 1.9(f) & 1.27(c) - SMALL BUSINESS CONCERN

I hereby declare that I am:

- the owner of the small business concern identified below;
 an official of the small business concern empowered to act on behalf of the concern identified below:

Name of Concern: GenVec, Inc.

Address of Concern: 65 West Watkins Mill Road
Gaithersburg, Maryland 20878

I hereby declare that the above-identified small business concern qualifies as a small business concern as defined in 13 C.F.R. § 121.3-18, and reproduced in 37 C.F.R. § 1.9(d), for purposes of paying reduced fees under Sections 41(a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement: (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time, or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either directly or indirectly one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention entitled: METHOD OF ENHANCING BONE DENSITY, by the inventors Ronald G. Crystal, Chisa Hidaka, Oheneba Boachie-Adjei, Bernard A. Rawlins, and Imre Kovesdi, as described in:

- The specification filed herewith.
 Application No. , filed .
 Patent No. , issued .

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Others Having Rights In The Invention

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I hereby declare that all statements made herein of my own knowledge are true, that all statements made on information and belief are believed to be true, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Name of Person Signing: Paul H. Fischer
Title in Organization: President and CEO
Address of Person Signing: 65 West Watkins Mill Road
Gaithersburg, MD 20878

Paul H. Fischer
Signature

July 31, 2000

Date

INVENTOR INFORMATION

Inventor One Given Name:: Ronald G
Family Name:: Crystal
Postal Address Line One:: 13712 Canal Vista Court
City:: Potomac
State or Province:: MD
Postal or Zip Code:: 20854
City of Residence:: Potomac
State or Province of Residence:: MD
Citizenship Country:: U.S.
Inventor Two Given Name:: Chisa
Family Name:: Hidaka
City:: New York
State or Province:: NY
City of Residence:: New York
State or Province of Residence:: NY
Inventor Three Given Name:: Oheneba
Family Name:: Boachie-Adjei
City:: New York
State or Province:: NY
City of Residence:: New York
State or Province of Residence:: NY
Inventor Four Given Name:: Bernard A
Family Name:: Rawlins
City:: New York
State or Province:: NY
City of Residence:: New York
State or Province of Residence:: NY
Inventor Five Given Name:: Imre
Family Name:: Kovesdi
Postal Address Line One:: 7713 Warbler Lane
City:: Rockville
State or Province:: MD
Postal or Zip Code:: 20855
City of Residence:: Rockville
State or Province of Residence:: MD
Citizenship Country:: Canada/U.S.

CORRESPONDENCE INFORMATION

Correspondence Customer Number:: 000023460
Fax One:: 312-616-5700
Electronic Mail One:: PPhillips@leydig.com
Electronic Mail Two:: MDHefner@leydig.com

APPLICATION INFORMATION

Title Line One:: METHOD OF ENHANCING BONE DENSITY

Formal Drawings?:: No
Application Type:: Utility
Docket Number:: 205965
Secrecy Order in Parent Appl.?:: No

REPRESENTATIVE INFORMATION

Representative Customer Number:: 23460

Source:: PrintEFS Version 1.0.1

METHOD OF ENHANCING BONE DENSITY OR FORMATION

TECHNICAL FIELD OF THE INVENTION

This invention pertains to a method and reagents for enhancing bone density or
5 formation.

BACKGROUND OF THE INVENTION

Most attempts of enhancing bone density or formation have traditionally come in
10 the form of increased support and/or the addition of bone graft material to the site of
treatment. Such approaches, however, have had only limited success and often fail to
provide aid to patients with bone healing deficiencies. For example, spinal fusion
15 protocols typically employ bone autografts, which are fractured into small pieces and
placed between the spinal processes to be fused. Such procedures achieve favorable
results only in about 40 % of treated patients, and the procedures for harvesting graft
material render an already invasive procedure even more so.

Efforts to mimic and/or supplement the normal series of events underlying proper
bone healing, and also to cure deficiencies associated with these events, have been
forthcoming. For example, blood vessel growth has been stimulated in normally healing
20 rabbit mandibular bones by mixing rabbit bone graft material *ex vivo* with basic fibroblast
growth factor (bFGF) and endothelial cells prior to graft implantation (Eppley *et al.*, *J.*
Oral Maxillofac. Surg., 46, 391-98 (1988)). Moreover, in efforts to accelerate fracture
healing, osteoblasts and osseous tissue have been infected *in vitro* and *in vivo* with vectors
25 delivering DNA encoding osteogenic proteins, such as transforming growth factor- β 1 and
bone morphogenic protein-2 (Baltzer *et al.*, *Gene Ther.*, 7, 734-79 (2000); Boden *et al.*,
Spine, 23, 2486-92 (1998); Gosdstein *et al.*, *Clin. Orthopaed. Rel. Res.*, 355S, S154-62
(1998); Mehrara *et al.*, *J. Bone Min. Res.*, 14(8), 1290-1300 (1999); Riew *et al.*, *Calcif.*
Tissue Int., 63, 357-60 (1998)). However, many such proteins precipitate an inhibitory
30 effect in treated tissues, and some discourage essential neovascularization within such
tissues. Moreover, such protocols requiring treatment of rare cells, such as stem cells,
depend on the isolation of sufficient quantities of such proteins, which can add yet another
level of invasiveness to the procedure, increasing morbidity and post-operative pain and
discomfort. Thus, despite improvements in the clinical treatment of bone injuries, there
continues to exist a need for improved compositions and/or methods that enhance bone
density or formation.

BRIEF SUMMARY OF THE INVENTION

One aspect of the invention pertains to a method for enhancing bone density or formation. In accordance with the method, a nucleic acid encoding an angiogenic protein is administered to a cell in a region of a bone such that the nucleic acid is expressed to 5 produce the angiogenic protein, whereby bone density or formation is enhanced within the region. Optionally, a nucleic acid encoding an osteogenic protein is administered to a cell within the same region such that the nucleic acid is expressed to produce the osteogenic protein. The method can be employed to produce a bone graft having a cell harboring an exogenous nucleic acid encoding an angiogenic protein and, optionally, a cell harboring a 10 nucleic acid encoding an osteogenic protein. To facilitate the inventive method, the invention provides a recombinant viral vector having a nucleic acid encoding an angiogenic protein and a nucleic acid encoding an osteogenic protein. These and other advantages, as well as additional inventive features, will become apparent after reading 15 the following detailed description.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the inventive method, a nucleic acid is administered to a cell (e.g., at least one cell) associated with a desired region of a bone. The relevant “region” of the bone includes the bone itself as well as the immediately adjoining area within the 20 bone or in tissues surrounding it (e.g., periosteum, muscle, fascia, tendons, ligaments, etc.). With this in mind, a cell is “associated with” the bone if it is within the region of the bone before, during, or following application of the inventive method. Any cell associated with the region of the bone can be treated in accordance with the inventive 25 method to express exogenous nucleic acids to produce (and typically secrete) encoded proteins. Inasmuch as such cells are employed as bioreactors in the application of the method, the type of cell is not critical. Thus the cell generally is any cell type associated with bony structures. Thus, for example, the cell can be within the bone (e.g., a preosteocyte, an osteocyte, chondrocyte, stromal cell, etc.) or in other tissue adjoining the 30 desired region (e.g., a periosteal or fascial cell, a muscle cell, etc.). Alternatively, a cell associated with the bone region can be initially away from the region and introduced into it during application of the method. For example, the cell can be within an exogenous tissue, such as a bone graft or other similar tissue, which is implanted or engrafted into the region of the bone.

The inventive method involves administering a nucleic acid (i.e., a first nucleic 35 acid) encoding an angiogenic protein to a cell (i.e., a first cell) within the region of the bone. An angiogenic protein is any protein that potentiates or enhances neovascularization, many of which are known in the art. While any such factor can be employed in the context of the inventive method, because VEGF proteins are not known

to induce the growth of tissues not involved in the production of new vasculature, a preferred angiogenic protein is a VEGF protein (e.g., VEGF_A, VEGF_B, VEGF_C, VEGF_D, VEGF_E), and more preferably VEGF₁₂₁, VEGF_{A138}, VEGF₁₄₅, VEGF_{A162}, VEGF₁₆₅, VEGF₁₈₂, VEGF₁₈₉, or a derivative thereof, (see, e.g., U.S. Patents 5,332,671 (Ferrara *et al.*), 5,240,848 (Keck *et al.*); and 5,219,739 (Tischer *et al.*)). Most preferably, because of their higher biological activity, the angiogenic protein is VEGF₁₂₁ or VEGF₁₆₅, particularly VEGF₁₂₁. Inasmuch as VEGF₁₂₁ typically binds heparin with lesser affinity than does VEGF₁₆₅, VEGF₁₂₁ is particularly preferred for use in the inventive method. While VEGF proteins are preferable for use in the inventive method, other angiogenic proteins include connective tissue growth factor (CTGF), VEGF2, VEGF-C, fibroblast growth factors (FGFs) (e.g., aFGF, bFGF, and FGF-4), angiopoietins, angiopoietin homologous proteins, angiogenin, angiogenin-2, and P1GF (see, e.g., U.S. Patents 5,194,596, 5,219,739, 5,338,840, 5,532,343, 5,169,764, 5,650,490, 5,643,755, 5,879,672, 5,851,797, 5,843,775, and 5,821,124; International Patent Application WO 95/24473; European Patent Documents 476 983, 506 477, and 550 296; Japanese Patent Documents 1038100, 2117698, 2279698, and 3178996; and J. Folkman *et al.*, *A Family of Angiogenic Proteins, Nature*, 329, 671 (1987)).

To enhance the efficacy of the inventive method, a nucleic acid (i.e., second nucleic acid) encoding an osteogenic protein can be similarly delivered to a cell (i.e., a second cell) within the same region of the bone. An osteogenic protein is any protein that potentiates or enhances ossification or differentiation of bone, many of which are known in the art. Osteogenic proteins include, for example, systemic hormones, (e.g., parathyroid hormone (PTH) estrogen, etc.), growth factors (e.g., CTGF and CTGF-like growth factor), cytokines, chemotactic and adhesive proteins, molecules such as activin (U.S. Patent 5,208,219), bone morphogenic proteins (BMPs), growth factor receptors, and the like. Preferably, the osteogenic protein of the present invention is selected a bone morphogenic protein (BMP) (e.g., BMP-2, BMP-3, BMP-4, BMP-5, BMP-6, BMP-7 and BMP-8), a transforming growth factor (TGF) (e.g., TGF- β 1), a latent TGF binding protein (LTBP), latent membrane protein-1 (LMP-1), a heparin-binding neurotrophic factor (HBNT), growth and differentiation factor-5 (GDF-5), a parathyroid hormone (PTH), a fibroblast growth factor (FGF), an epidermal growth factor (EGF), a platelet-derived growth factor (PDGF), an insulin-like growth factor (e.g., IGF-1 or 2)), a growth factor receptor, a cytokine, a chemotactic factor, a granulocyte/macrophage colony stimulating factor (GMCSF), a LIM mineralization protein (LMP) (Boden *et al.*, *Spine*, 23, 2486-92 (1998)), a leukemia inhibitory factor (LIF), a hedgehog protein (e.g., Desert Hedgehog (DHH), Indian Hedgehog (IHH), Sonic Hedgehog, etc.) or a derivative thereof. Most preferably, the osteogenic protein is TGF- β 1 or midkine (MK) and, as discussed above, these are preferably employed where the angiogenic factor is a VEGF. Some

osteogenic proteins also can stimulate the growth or regeneration of skeletal connective tissues such as, e.g., tendon, cartilage, ligament, etc.

While the sequences of many angiogenic and osteogenic proteins, and nucleic acids encoding them, are known, any active derivative sequence can be employed in the place of known sequences. These derivatives include those caused by point mutations, those due to the degeneracies of the genetic code or naturally occurring allelic variants, and further modifications that have been introduced by genetic engineering.

Where a nucleic acid encoding an angiogenic protein and a nucleic acid encoding an osteogenic protein (i.e., first and second nucleic acids) are both employed in the inventive method, they can be delivered to the same or different cells associated with the region of the bone. In this respect the first cell and the second cell can be the same cells or different cells. The method is more efficacious if the nucleic acid(s) are delivered to many cells associated with the region of the bone, such as a population of cells, or a majority of cells within the region. In any event, within at least the first cell, the first nucleic acid is expressed, leading to the production of the angiogenic protein. Desirably, a second nucleic acid is employed to similarly lead to the production of an osteogenic protein. Typically the encoded protein(s) is secreted from the cell, but it need not be. The presence of the angiogenic (and desirably osteogenic) protein promotes physiological changes within the region of the bone so as to enhance bone density or formation.

Successful application of the inventive method enhances bone density or formation in any respect. Thus, it is to be understood that the inventive method can strengthen or harden a region of contiguous bone. In other applications, enhancement of bone density or formation is associated with healing (e.g., fusion) of splintered or fractured bone.

Similarly, the inventive method can facilitate fusion of two bone masses, such as a bone graft to a bony region within a patient or the fusion of separate bones within a patient, such as vertebrae or other desired bony structures. Moreover, in certain embodiments, the inventive method can be employed to stimulate the growth or repair of both bone tissue itself and also of skeletal connective tissues that surround or are associated with bone. Thus, the method can facilitate the attachment of such bone-associated tissues (e.g., ligaments) to bones.

A nucleic acid employed in the inventive method can be any suitable type sufficient to lead to the production of the desired protein within the cell(s) associated with the desired region of bone. In this respect, a nucleic acid can be RNA, cDNA, genomic DNA, etc., but typically it is cDNA, such as, for example, within an expression cassette.

Moreover, in embodiments in which a polynucleotide encoding an osteogenic protein is delivered in conjunction with the nucleic acid encoding the angiogenic protein, the two nucleic acids can be present in the same molecule or on separate molecules (i.e., the first nucleic acid and the second nucleic acid can be the same). Of course, inasmuch as these

nucleic acids can be delivered to different cells (i.e., first and second cells), it is quite possible for the two coding nucleic acids to be present on separate molecules.

Where a nucleic acid for use in the inventive method is within an expression cassette, the cassette also should have promoter able to drive the expression of the coding sequence within the cells. Many viral promoters are appropriate for use in such an expression cassette (e.g., retroviral ITRs, LTRs, immediate early viral promoters (IEp) (such as herpesvirus IEp (e.g., ICP4-IEp and ICP0-IEp) and cytomegalovirus (CMV) IEp), and other viral promoters (e.g., late viral promoters, latency-active promoters (LAPs), Rous Sarcoma Virus (RSV) promoters, and Murine Leukemia Virus (MLV) promoters)). Other suitable promoters are eukaryotic promoters, such as enhancers (e.g., the rabbit β -globin regulatory elements), constitutively active promoters (e.g., the β -actin promoter, etc.), signal specific promoters (e.g., inducible and/or repressible promoters, such as a promoter responsive to TNF or RU486, the metallothionein promoter, etc.), and tissue-specific promoters. Moreover, where the first and second nucleic acids are part of the same molecule, their respective cassettes can share a bi-directional promoter, many of which are known in the art (see, e.g., Lee *et al.*, *Mol Cells.*, 10(1), 47-53 (2000), Dong *et al.*, *J. Cell. Biochem.*, 77(1), 50-64 (2000), and Li *et al.*, *J. Cell. Biochem.*, 273(43), 28170-77 (1998)), such that the respective coding sequences can be on opposite strands of the molecule.

Regardless of the type of promoter employed, within the expression cassette, the coding polynucleotide and the promoter are operably linked such that the promoter is able to drive the expression of the desired sequence. As long as this operable linkage is maintained, the expression cassette can include more than one gene, such as multiple coding sequences (e.g., the first and the second nucleic acids, as discussed herein) separated by ribosome entry sites. Furthermore, the expression cassette can optionally include other elements, such as polyadenylation sequences, transcriptional regulatory elements (e.g., enhancers, silencers, etc.), or other sequences.

For successful application of the inventive method, a nucleic acid encoding the angiogenic and/or the osteogenic protein must be introduced into a cell associated with the desired region of the bone in a manner suitable for it to express the encoded sequence. Any suitable vector can be employed to this end, many of which are known in the art. Examples of such vectors include naked RNA and DNA vectors (such as oligonucleotides, artificial chromosomes (e.g., yeast artificial chromosomes (YACs)), cosmids, plasmids, etc.), viral vectors such as adeno-associated viral vectors (Berns *et al.*, *Ann. N.Y. Acad. Sci.*, 772, 95-104 (1995)), adenoviral vectors (Bain *et al.*, *Gene Therapy*, 1, S68 (1994)), herpesvirus vectors (Fink *et al.*, *Ann. Rev. Neurosci.*, 19, 265-87 (1996), U.S. Patents 5,837,532; 5,846,782; 5,849,572; and 5,804,413 and International Patent Applications WO 91/02788, WO 96/04394, WO 98/15637, and WO 99/06583), packaged

amplicons (Federoff *et al.*, *Proc. Nat. Acad. Sci. USA*, 89, 1636-40 (1992)), papilloma virus vectors, phage vectors, picornavirus vectors, polyoma virus vectors, retroviral vectors, SV40 viral vectors, vaccinia virus vectors, and other vectors. While some of the indicated vectors are suitable for use only with certain types of polynucleotides (e.g.,

5 cDNA as opposed, for example, to RNA), the selection of an appropriate vector and the use thereof to introduce exogenous genetic material (e.g., the desired nucleic acids) into cells are within the skill of the art. Once a given type of vector is selected, its genome must be manipulated for use as a background vector, after which it must be engineered to incorporate exogenous polynucleotides. Methods for manipulating the genomes of
10 vectors are well known in the art (see, e.g., Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, 2d edition, Cold Spring Harbor Press (1989); Ausubel *et al.*, *Current Protocols in Molecular Biology*, Greene Publishing Associates and John Wiley & Sons, New York, N.Y. (1994)) and include direct cloning, site specific recombination using recombinases, homologous recombination, and other suitable methods of constructing a
15 recombinant vector. In this manner, an expression cassette can be inserted into any desirable position of the vector. Moreover, in addition to the desired expression cassette, a vector also can include other genetic elements as appropriate, such as, for example, genes encoding a selectable marker (e.g., β -gal or a marker conferring resistance to a
20 toxin, such as puromycin or other similar selectable markers), a pharmacologically active protein, a transcription factor, or other biologically active substance.

As mentioned, within the context of the inventive method, one or more of the nucleic acids can be delivered to the cells as (i.e., within) a viral vector. To facilitate such embodiments of the method, the invention provides a viral vector having a first nucleic acid encoding an angiogenic protein and a second nucleic acid encoding an osteogenic protein, such as the angiogenic and osteogenic proteins discussed herein. While the inventive viral vector can be any suitable type of virus, adenoviral vectors present several advantages, particularly for *in vivo* applications, not the least of which is that the knowledge of such vector has advanced to a stage where virulence can be eliminated, tropism can be altered, exogenous genetic material can be introduced into such viral
25 backbone, and the virus can be efficiently constructed, grown, purified, and stored (see, e.g., U.S. Patents 6,063,627, 6,057,155, 6,013,638, 5,997,509, 5,994,106, 5,965,541, 5,965,358, 5,962,311, 5,928,944, 5,869,037, 5,851,806, 5,849,561, 5,846,782, 5,837,511, 5,801,030, 5,770,442, 5,731,190, 5,712,136, and 5,559,099; International Patent Applications WO00/34496, WO00/34444, WO00/23088, WO00/15823, WO00/12765,
30 WO00/00628, WO99/55365, WO99/54441, WO99/41398, WO99/23229, WO99/15686, WO98/56937, WO98/54346, WO98/53087, WO98/40509, WO98/32859, WO98/07877, WO98/07865, WO97/49827, WO97/21826, WO97/20051, WO97/12986, WO97/09439 and WO 96/26281, WO 96/07734, WO 95/34671; and European Patent Documents

0863987, 0866873, 0870049, 0914459, 0920524, 0973927, 0988390, 0996735, 1012291, 1015620). Indeed, recombinant adenoviruses having angiogenic genes are known in the art (see, e.g., Mack *et al.*, *J. Thorac. Cardiovasc. Surg.*, 115(1), 168-76 (1998); Magovern *et al.*, *Hum. Gene. Ther.*, 8(2), 215-27 (1997)), and a nucleic acid encoding an 5 osteogenic protein can be cloned into such a backbone vector by standard methods.

Given the state of the art, an adenoviral vector of the present invention can be derived from any desired serotype of adenovirus. Adenoviral stocks that can be employed as a source of adenovirus can be amplified from the adenoviral serotypes 1 through 51, which are currently available from the American Type Culture Collection (ATCC, 10 Rockville, MD), or from any other serotype of adenovirus available from any other source. For instance, an adenovirus can be of subgroup A (e.g., serotypes 12, 18, and 31), subgroup B (e.g., serotypes 3, 7, 11, 14, 16, 21, 34, and 35), subgroup C (e.g., serotypes 1, 2, 5, and 6), subgroup D (e.g., serotypes 8, 9, 10, 13, 15, 17, 19, 20, 22-30, 32, 33, 36-39, 15 and 42-47), subgroup E (serotype 4), subgroup F (serotypes 40 and 41), or any other adenoviral serotype. Preferably, however, an adenovirus is of serotype 2, 5 or 9.

Typically, aside from containing the sequences encoding the osteogenic and angiogenic proteins, the viral vector is deficient in at least one essential gene function. Such manipulations generally render the vector unable to replicate except in cells engineered to provide the missing essential gene function(s). For example, an adenoviral vector can have at least a partial deletion of the E1 (e.g., E1a or E1b), E2 and/or E4 regions, and desirably such a virus has a deletion in two, three or even all of these regions. Suitable replication-deficient adenoviral vectors are disclosed in U.S. Patents 5,851,806 and 5,994,106 and International Patent Applications WO 95/34671 and WO 97/21826. Indeed, in preferred embodiments, at least one of the exogenous nucleic acids (e.g., 25 encoding the osteogenic or the angiogenic proteins) is cloned into the E1 region of the adenoviral backbone, desirably oriented from "right to left" within the genome. While not essential for viral replication, the inventive adenoviral vector also can have at least a partial deletion in the E3 region as well.

In addition to a deficiency in the E1, E2, E3, and/or E4 region, an adenoviral 30 vector according to the invention also can have a mutation in the major late promoter (MLP), for example in one or more control element(s) such that it alters the responsiveness of the promoter. Moreover, the tropism of viral vectors can be altered, for example by incorporating chimeric coat proteins into a viral surface that contains ligands able to mediate viral attachment to novel cell surfaces (e.g., either directly or through a bi- 35 or multi-specific molecule) and/or by destroying the native tropism of the virus. Where the tropism of the virus is altered from that of the source virus, preferably it is engineered to contain a ligand conferring the ability to bind cells associated with bone tissue, such as, for example, osteocytes, chondrocytes, periosteal cells, myocytes, and cells in muscle and

tendons that are associate with the type of bone to be treated. Many such ligands are known, and techniques for generating replication deficient adenoviral vectors and for altering viral tropism are well known in the art.

In application, the first and/or second nucleic acids (or a virus containing them, if

5 appropriate) are delivered to the cell within a physiologically-acceptable solution.

Accordingly, to facilitate the inventive method, the invention provides a pharmaceutical (including pharmacological) composition including a first nucleic acid encoding an angiogenic protein, a second nucleic acid encoding an osteogenic protein (which can, of course, be within a recombinant virus as described herein), and a diluent. The diluent can

10 include one or more pharmaceutically- (including pharmacologically- and physiologically-) acceptable carriers. For compositions suitable for *in vitro* application, the diluent can be a suitable tissue culture medium. Pharmaceutical compositions for use in accordance with the present invention can be formulated in any conventional manner

using one or more pharmaceutically- or physiologically-acceptable carriers comprising excipients, as well as optional auxiliaries which facilitate processing of the nucleic acids into preparations which can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen. Thus, for systemic injection, the nucleic acids can be formulated in aqueous solutions, preferably in physiologically-compatible buffers.

The nucleic acids can be formulated for parenteral administration by injection, e.g., by bolus injection or continuous infusion. Such compositions can take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and can contain formulatory agents such as suspending, stabilizing and/or dispersing agents. For application to bony tissues, a preferred composition includes a porous or spongy matrix, such as collagen, which can be soaked or perfused with a fluid or semifluid carrier (e.g.,

25 buffered saline solution) including the nucleic acid(s). Such a matrix material assists in retaining the nucleic acid(s) within the site of the bone to be treated. Of course, the nucleic acids also can be formulated into other compositions appropriate to the vector type such as those known in the art. Thus, for example, the nucleic acids could be administered in combination with further agents, such as, e.g., liposomes, lipids (e.g.,

30 cationic or anionic lipids), polypeptides, or various pharmaceutically active agents. In some embodiments, the nucleic acids can be delivered along with various other agents, such as an angiogenic factor and/or an inhibitor of bone resorption (see, e.g., U.S. Patents 5,270,300 and 5,118,667).

The composition(s) containing the nucleic acids (or viral vector containing them)

35 is delivered to tissue associated with the region of bone to be treated at any dose appropriate to enhance bone density or formation within the region. The appropriate dose will vary according to the type of vector employed, but it is within routine skill to select an suitable dosage. Thus, for example, where the nucleic acids are within an adenoviral

vector, a dose typically will be at least about 1×10^5 pfu (e.g., 1×10^6 - 1×10^{12} pfu) to the site of administration. The dose preferably is at least about 1×10^7 pfu (e.g., about 1×10^7 - 1×10^{12} pfu), more preferably at least about 1×10^8 pfu (e.g., about 1×10^8 - 1×10^{11} pfu), and most preferably at least about 1×10^9 pfu (e.g., about 1×10^9 - 1×10^{10} pfu). For purposes of
5 considering the dose in terms of particle units (pu), also referred to as viral particles, typically within about an order of magnitude of from about 10 to about 100 particles is equivalent to about 1 pfu (e.g., 1×10^{12} pfu is roughly equivalent to 1×10^{14} pu). In a single
10 round of vector administration, using, for example, an adenoviral vector deleted of the E1a region, part of the E1b region, and part of the E3 region of the adenoviral genome,
15 wherein the vector carries human VEGF₁₂₁ or VEGF₁₆₅ under the control of a standard CMV immediate early promoter, about 10^7 - 10^{12} pfu, preferably about 10^9 - 10^{10} pfu, are administered to the desired region. Of course, the amount of virus administered can vary depending on the volume of the area to be treated.

15 The composition is administered to the region of the bone in an appropriate manner to deliver the nucleic acids to the tissue. For application *in vivo*, the region of the bone is exposed, and the composition is delivered, so as to be in physical contact with the tissues within the region. While in some applications it is desirable to expose the bone itself, in other applications tissue surrounding or otherwise associated with the bone can be retained intact, with the composition delivering the nucleic acids to cells existing within such tissues. Inasmuch as the method can be employed in conjunction with standard surgical techniques, its application can be directed to serve any desired treatment goal. For example, the method can be employed to promote fracture repair by delivering the composition to the region of the fracture. Alternatively, the method can be employed with methods for bone fusion (e.g., vertebral fusion).

20 25 For application *in vitro*, such as on a bone graft, the bone material can be bathed in a composition containing the nucleic acids or, where appropriate, the bone material can be perfused with the composition. The period of such bathing or perfusion should be sufficient so as to permit the cell or cells to take up the nucleic acids. Depending on the desired use of the graft and the genetic constructs employed (e.g., inducible promoters, etc.), the cells need not be induced to express the nucleic acids *in vitro*.

30 Where the method is employed *in vitro*, it can be used to create bone grafts for tissue repair. Accordingly, the invention provides a bone graft having a first cell (preferably a population of cells) having a first exogenous nucleic acid encoding an angiogenic protein and optionally a second cell (preferably a population of cells) having a second nucleic acid encoding an osteogenic protein, such as are set forth above. Within 35 the graft, the first and second cells can be the same, as can the first and second nucleic acids. The graft can be obtained from any suitable donor source according to commonly employed surgical techniques, the iliac crest being a common source of tissue for bone

grafts. Alternatively, the graft can be grown *de novo* (e.g., from osteocytes, preosteocytes, stem cells, cartilage, etc.) prior to treatment. In this respect, the graft can be an autograft, derived from any desirable bony structure in the patient to whom the graft is to be re-implanted. In other applications, the graft can be an allograft or even a 5 xenograft. Indeed, such graft tissue can be preserved for use in future applications, e.g., through incubation in culture medium, refrigeration, cryopreservation, etc. In any event, after the nucleic acids have been transferred to a cell or cells within the graft, it is implanted into a patient according to standard surgical techniques. Where the graft is other than an autograft, however, appropriate immunosuppression should be employed as 10 necessary to mitigate graft rejection. The cell(s) within the graft to which the nucleic acids have been transferred should express the nucleic acids to produce the angiogenic and osteogenic proteins at least after the graft has been implanted into the patient. As discussed, the presence of such proteins within the region of the fissure between the graft and the host tissue will facilitate fusion of the graft to the host bone.

EXAMPLE

While one of skill in the art is fully able to practice the instant invention upon reading the foregoing detailed description, the following example will help elucidate some of its features. In particular, it demonstrates that the transfer of a nucleic acid encoding an angiogenic or osteogenic protein to cells associated with a region of bone can increase bone density or formation and facilitate fusion of separate bony structures. As this example is presented for purely illustrative purposes, it should not be used to construe the scope of the invention in a limited manner, but rather it should be seen as expanding upon the foregoing description of the invention as a whole.

The procedures described in this example were conducted on 250 g Sprague-Dawley rats. Recombinant adenoviral vectors were constructed having either the *E. coli* β-galactosidase gene (Adβgal) or the VEGF₁₂₁ or VEGF₁₆₅ isoforms (AdVEGF₁₂₁ and AdVEGF₁₆₅). All adenovirus vectors were E1⁻, partial E3⁻ and based on the Ad5 genome, with transgenes in the E1 position under the CMV promoter/enhancer. (Mack *et al.*, *J. Thorac. Cardiovasc. Surg.*, 115(1), 168-76 (1998); Magovern *et al.*, *Hum. Gene. Ther.*, 8(2), 215-27 (1997)).

Single level posterior lumbar arthrodesis was attempted in 30 rats. Left and right L4 and L5 transverse processes were exposed through a paraspinal muscle-splitting approach and then decorticated using scalpel blade. HELISTAT (COLLA-TEC, 35 Plainsboro, NJ, USA) bovine collagen sponges were soaked with saline alone (75μl/site) or saline with the various adenoviral vectors (10¹⁰ particle units/site). The treated sponges were inserted between the decorticated L4 and L5 transverse processes on both left and right sides.

In one experiment, conducted on 30 rats, three rats received no graft, 5 received saline alone, 6 received Ad β gal, 7 received AdVEGF₁₂₁, 6 received AdVEGF₁₆₅, and 3 received a preparation of recombinant VEGF₁₆₅ (5 μ l/side, R&D, Minneapolis, MN, USA). In a second experiment, three groups of three rats each were treated by the

5 AdVEGF₁₂₁ or AdVEGF₁₆₅, Ad β gal, or saline. In each experiment, the spines were harvested 4 wks post-operatively and evaluated by gross inspection, high-resolution radiographs, and microscopic examination of H&E-stained decalcified histological specimens. Radiographs were assessed by three blinded observers.

In the first experiment, upon gross inspection new bone formation was evident in 4

10 sites (29% of sites) treated with AdVEGF₁₂₁, 4 sites (33% of sites) treated with AdVEGF₁₆₅. No gross evidence of bone was noted in spines treated with no carrier, no virus (saline alone), control virus (Ad β gal) or recombinant VEGF protein. Radiographs revealed an increase in the formation of radio-dense bone between L4 and L5 transverse processes in spines treated with AdVEGF_(121 or 165) when compared to controls.

15 Specimens with radiographic evidence of new bone formation also showed the presence of tissue with the histological appearance of new woven bone was contiguous with the L4 or L5 transverse processes. In the second experiment, 3 sites (50% of sites) treated with the AdVEGF vectors showed the presence of bone growth between the processes, while no bone growth was observed with the Ad β gal or saline controls. Similar results (50% of treated sites) were observed in animals treated with an adenoviral vector having the coding sequence encoding bone morphogenic protein-1 (AdBMP).

INCORPORATION BY REFERENCE

All sources (e.g., inventor's certificates, patent applications, patents, printed

25 publications, repository accessions or records, utility models, world-wide web pages, and the like) referred to or cited anywhere in this document or in any drawing, Sequence Listing, or Statement filed concurrently herewith are hereby incorporated into and made part of this specification by such reference thereto.

30

Interpretation Guidelines

The foregoing detailed description sets forth "preferred embodiments" of this invention, including the best mode known to the inventors for carrying it out. Of course, upon reading the foregoing description, variations of those preferred embodiments will become obvious to those of ordinary skill in the art. The inventors expect skilled artisans 35 to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law.

As used herein, singular indicators (e.g., "a" or "one") include the plural, unless otherwise indicated. The term "consisting essentially of" indicates that unlisted ingredients or steps that do not materially affect the basic and novel properties of the invention can be employed in addition to the specifically recited ingredients or steps. In
5 contrast, the terms "comprising" or "having" indicate that any ingredients or steps can be present in addition to those recited. The term "consisting of" indicates that only the recited ingredients or steps are present, but does not foreclose the possibility that equivalents of the ingredients or steps can substitute for those specifically recited.

WHAT IS CLAIMED IS:

1. A method for enhancing bone density or formation, the method comprising administering to at least one first cell associated with a region of a bone at least one first nucleic acid encoding at least one angiogenic protein, such that the first nucleic acid is expressed in the cell to produce the angiogenic protein, whereby bone density or formation is enhanced within the region.

5 2. The method of claim 1, wherein at least one of the nucleic acids is exposed to at least one cell *in vivo* in the region of the bone.

10 3. The method of claim 1, wherein at least one of the nucleic acids is exposed to at least one cell *ex vivo*, which is then delivered *in vivo* to the region of the bone.

15 4. The method of claim 1, wherein the angiogenic protein is a vascular endothelial growth factor (VEGF), a connective tissue growth factor (CTGF), VEGF2, VEGF-C, a fibroblast growth factor (FGF), an angiopoietin, an angiopoietin homologous proteins, an angiogenin, an angiogenin-2, P1GF, or a derivative thereof.

20 5. The method of claim 1, wherein the angiogenic protein is selected from the group consisting of VEGF₁₂₁, VEGF_{A138}, VEGF_{A162}, VEGF₁₆₅, VEGF₁₈₂, VEGF₁₈₉, and derivatives thereof.

25 6. The method of claim 1, further comprising administering to at least one second cell associated with the region at least one second nucleic acid encoding at least one osteogenic protein, such that the second nucleic acid is expressed in the cell to produce the osteogenic protein.

30 7. The method of claim 6, wherein the osteogenic protein is selected from the group consisting of a bone morphogenic protein (BMP), a transforming growth factor (TGF), a latent TGF binding protein (LTBP), latent membrane protein-1 (LMP-1), a heparin-binding neurotrophic factor (HBNF), growth and differentiation factor-5 (GDF-5), a parathyroid hormone (PTH), a fibroblast growth factor (FGF), an epidermal growth factor (EGF), a platelet-derived growth factor (PDGF), an insulin-like growth factor, a growth factor receptor, a cytokine, a chemotactic factor, a granulocyte/macrophage colony stimulating factor (GMCSF), a LIM mineralization protein (LMP), a leukemia inhibitory factor (LIF), a hedgehog protein, midkine (MK), and derivatives thereof.

35 8. The method of claim 6, wherein the osteogenic protein is selected from the group consisting of BMP-2, BMP-3, BMP-4, BMP-5, BMP-6, BMP-7 and BMP-8.

9. The method of claim 6, wherein the osteogenic protein is TGF- β 1.

10. The method of claim 6, wherein the osteogenic protein is BMP-2.

35 11. The method of claim 6, wherein the osteogenic protein is MK.

12. The method of claim 6, wherein the osteogenic protein is HBNF.

13. The method of claim 6, wherein the angiogenic protein is a VEGF, and the osteogenic protein is TGF- β 1.

14. The method of claim 6, wherein the angiogenic protein is a VEGF, and the osteogenic protein is BMP-2.

15. The method of claim 6, wherein the angiogenic protein is a VEGF, and the osteogenic protein is MK.

5 16. The method of claim 6, wherein the angiogenic protein is a VEGF, and the osteogenic protein is HBNF.

17. The method of claim 6, wherein the first cell and the second cell are the same.

18. The method of claim 6, wherein the first nucleic acid and the second
10 nucleic acid are the same.

19. A viral vector comprising at least one first nucleic acid encoding at least one angiogenic protein and at least one second nucleic acid encoding at least one osteogenic protein.

20. The viral vector of claim 19, which is an adenoviral vector.

15 21. The viral vector 19, which is deficient in at least one essential gene function.

22. A bone graft comprising at least one first cell having at least one first exogenous nucleic acid encoding at least one angiogenic protein and at least one second cell having at least one second nucleic acid encoding at least one osteogenic protein.

20 23. The bone graft of claim 19, wherein the osteogenic protein is selected from the group consisting of a bone morphogenic protein (BMP), a transforming growth factor (TGF), a latent TGF binding protein (LTBP), latent membrane protein-1 (LMP-1), a heparin-binding neurotrophic factor (HBNF), growth and differentiation factor-5 (GDF-5), a parathyroid hormone (PTH), a fibroblast growth factor (FGF), an epidermal growth factor (EGF), a platelet-derived growth factor (PDGF), an insulin-like growth factor (IGF), a growth factor receptor, a cytokine, a chemotactic factor, a granulocyte/macrophage colony stimulating factor (GMCSF), a LIM mineralization protein (LMP), a leukemia inhibitory factor (LIF), a hedgehog protein, midkine (MK).and derivatives thereof.

25 24. The bone graft of claim 19, wherein the angiogenic protein is a vascular endothelial growth factor (VEGF).

30 25. The bone graft of claim 19, which is an allograft.

ABSTRACT

The present invention is directed to a method for enhancing bone density or formation. In accordance with the method, a nucleic acid encoding an angiogenic protein is administered to a cell in a region of a bone such that the nucleic acid is expressed to produce the angiogenic protein, whereby bone density or formation is enhanced within the region. Optionally, a nucleic acid encoding an osteogenic protein is administered to a cell within the same region such that the nucleic acid is expressed to produce the osteogenic protein. The method can be employed to produce a bone graft having a cell harboring an exogenous nucleic acid encoding an angiogenic protein and, optionally, a cell harboring a nucleic acid encoding an osteogenic protein. To facilitate the inventive method, the invention also pertains to a recombinant viral vector having a nucleic acid encoding an angiogenic protein and a nucleic acid encoding an osteogenic protein.

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